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EXAMINER

PORTNER, VIRGINIA ALLEN

ART UNIT

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1645

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14

Please find below and/or attached an Office communication concerning this application or proceeding.

File Copy

Office Action Summary

Application No.
09/848,909

Applicant(s)
Collier et al

Examiner
Portner

Art Unit
1645



-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on Sep 23, 2002
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-39 is/are pending in the application.
- 4a) Of the above, claim(s) 9-11, 21-28, 32, and 36-39 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-8, 12-20, 29-31, and 33-35 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☒ Claims 1-39 are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some* c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

- 14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). 8, 10
- 4) ☐ Interview Summary (PTO-413) Paper No(s). _____
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☒ Other: SEQUENCE LETTER

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DETAILED ACTION

Claims 1-39 are pending.

New claims 29-39 (37 CFR 1.126; claim 28 already pending in instant Application) have been submitted.

Election/Restriction

1. Claims 9-11, 21-28, 32 (dependent upon withdrawn claim 11), 32, 36-39 are herein withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected Groups II or III, there being no allowable generic or linking claim. Election was made without traverse in Paper No. 13, dated September 16, 2002. Elected species being D425K of SEQ ID No 1. Non-elected species are herein withdrawn from further consideration.
2. Claims 1-8, 12-20, 29-31, 33-35 are under consideration; claims 4 and 19 recite the elected species.

Sequence Letter

3. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. §§ 1.821-1.825 for the reason(s) set forth on the attached Notice To Comply With Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

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4. ☒ APPLICANT IS GIVEN the time period set for THIS LETTER WITHIN WHICH TO COMPLY WITH THE SEQUENCE RULES, 37 C.F.R. §§ 1.821-1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R. § 1.821(g). Extensions of time may be obtained by filing a petition accompanied by the extension

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fee under the provisions of 37 C.F.R. § 1.136. In no case may an applicant extend the period for response beyond the six month statutory period. Direct the response to the undersigned. Applicant is requested to return a copy of the attached Notice to Comply with the response.

Please Note: SEQ ID No 12 and 21 are identical. While this is clearly permissible, SEQ ID NO 12 is defined differently from SEQ ID NO. 21 in the instant specification. SEQ ID NO 12, is defined to evidence a specific deletion of amino acids 302-325, as shown in Table 1, page 20 of the specification, while SEQ ID NO 21 (defined at page 6, line 3) is defined to be the native sequence. Based upon the sequence listing provided by Applicant, both sequences (SEQ ID NO 12 and 21) do not differ one from the other as both sequences have 736 amino acids in them. The sequence listing should be in agreement with the disclosure of the instant specification and evidence the sequences as defined by the narrative used to describe Applicant's invention.

Information Disclosure Statement

5. The information disclosure statements filed January 25, 2002 and October 9, 2001 have been considered as to the merits.

Double Patenting

6. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

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A timely filed terminal disclaimer in compliance with 37 CFR 1.321© may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

7. Claims 1, 5-6, 12, 33 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 4-6, and 7 of U.S. Patent No. 6,455,673.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the allowed species anticipates the instantly claimed genus, wherein the allowed species evidences specific mutations in the receptor binding moiety of a binary A-B toxin; the allowed toxins being moieties evidencing less affinity for sensitive cell than the wild-type toxin (see US Pat. 6,455, 673, col. 2, lines 34-40 and lines 14-35).

8. Claims 1, 6, 8, 12, 15, 20, 33 (renumbered claim 32) are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-13, and 23-27 of U.S. Patent No. 5,917,017. Although the conflicting claims are not identical, they are not patentably distinct from each other because the allowed species anticipates the instantly claimed genus, wherein the allowed species evidences specific mutations in the receptor binding moiety of a binary A-B toxin; the allowed toxins being moieties evidencing less affinity for sensitive cell than the wild-type toxin, wherein a claimed species comprises a deletion of amino acids 379-535 which includes the deletion of a D2L2 Loop. The allowed species anticipates the instantly claimed genus.

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Claim Objections

9. The numbering of claims is not in accordance with 37 CFR 1.126 which requires the original numbering of the claims to be preserved throughout the prosecution. When claims are canceled, the remaining claims must not be renumbered. When new claims are presented, they must be numbered consecutively beginning with the number next following the highest numbered claims previously presented (whether entered or not). Misnumbered claims 28-38 been renumbered 29-39.

Claim Rejections - 35 U.S.C. § 112

10. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

11. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

12. Claims 1-3, 5-8, 12-18, 20, 29-31, 33-35 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant specification does not contain a written description of the invention in such full, clear, concise, and exact terms or in sufficient detail that one skilled in the art can reasonably conclude that applicant had possession of the claimed invention at the time of filing.

The claimed invention, is directed to B-moieties that have been mutated and do not comprise a mutation of amino acids 302-325 of SEQ ID NO 12, or is directed to any B-moieties, from any source, that comprise any mutation that would serve to inhibit pore forming ability,

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wherein the B-moieties would induce a protective immune response against challenge and serve as a vaccine, has not been described.

The specification discloses a mutant anthrax protective antigen B-moiety, with a D425N substitution, as well as other mutant anthrax protective antigen B-moieties which may evidence site specific alterations in the amino acid sequence. What the alterations are in the claimed plurality of mutant B-moieties, that share at least 80% sequence identity with SEQ ID No 21 or 12, comprise a D2L2 loop and is not anthrax toxin, and have any amino acid sequence, has not been described.

A description of a genus may be achieved by means of a recitation of a representative species, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus. (*Regents of the University of California v. Eli Lilly & Co.*, 119 F3d 1559, 1569, 43 USPQ2d 1398-1412, 1406 (Fed. Cir. 1997)).

The claimed mutant B-moieties, the products which do not have any specific function other than the negative functional limitation “inhibits pore forming ability” have not been described (instantly claimed independent claims 1, 6, 12).

The instant specification fails to provide sufficient descriptive information, such as definitive structural or functional features of the claimed genus mutant B-moieties that have alterations in any location resulting in inhibition of pore forming ability. The size, sequences, sources and positively recited biological activities relative to specific mutation B-moieties are not recited. Even if the claims were amended to require the mutant B-moiety to correspond to either SEQ ID No 12 or 21, the number and type of mutations for this genus also have not been described other than by specific mutations disclosed and claimed in claims 4 and 19. These specific mutations are relative to a molecule that can be 80% identical to SEQ ID No 21 and which molecule has not been described. A molecule that shares 80% identity with SEQ ID No 21

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would differ by up to 152 amino acids; where these changes are, and what the sequences are of the about 611 amino acid sequences have not been described.

There is no description of where or how the alterations in any B-moiety must be made to achieve or maintain the desired effect of induction of a protective immune response upon administration of the mutant B-moiety to a host. For example, *Pseudomonas aeruginosa* pore forming cytotoxin (see Swiss Prot accession number Q38644, shown to evidence 4 domains/moieties by Prosite) evidences 389 amino acids, while anthrax protective antigen has 764 (Swiss-Prot accession number P13423). How a mutation in the B-moiety of a 389 amino acid sequence for the pore forming toxin of *Pseudomonas*, relative to a mutation in anthrax toxin at position 427 has not been described.

The specification proposes to discover mutant B-moieties that would inhibit pore forming ability in other B-moieties other than those defined for anthrax protective antigen, what these proposed mutation are have not been described for the genus of B-moieties now claimed.

Vas-Cath Inc. V. Mahurkar, 19 USPQ2d 1111, clearly states that “applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of *the invention*. The invention is, for purposes of the ‘written description’ inquiry, *whatever is now claimed*.” (See page 1117). The specification does not “clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed.” (See *Vas-Cath* at page 1116). Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C. 112 is severable from its enablement provision (see page 115).

Reiger et al (Glossary of Genetics and Cytogenetics, Classical and Molecular, 4th Ed., Springer-Verlag, Berlin, 1976) clearly define alleles as one of two or more alternative forms of a gene occupying the same locus on a particular chromosome ... and differing from other alleles of that locus at one or more mutational sites (page 17). Thus, the structure of naturally occurring allelic sequences are not defined. Conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written

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description requires more than a mere statement that it is part of the invention and a reference to a potential method of isolating it. The nucleic acid itself is required. See *Fiers v. Revel*, 25 USPQ 2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. V. Chugai Pharmaceutical Co. Lts.*, 18 USPQ2d 1016.

Mutant B-moieties that evidence inhibited pore forming ability, and are any size fragment of SEQ ID NO 21 or 12, a homolog or allelic variant that shares a sequence with SEQ ID No 21 or 12 and is able to induce a protective immune response have not been described.

Sufficient support for the generic claims has not been provided. See the Interim Guidelines on Written Description, (Fed Reg , June 15, 1998, Volume 63, Number 114, pages 32639-32645) and the Revised Interim Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 64, No. 244, pages 71427-71440, Tuesday December 21, 1999.

13. Claims 6-8, 33-35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the production and use of an mutant anthrax toxin for induction of an immune response, as well as specific site directed mutants of PA antigen (PA63) for the induction of a protective immune response but, does not reasonably provide enablement for the use of any mutant toxin (protein or polypeptide) as a pharmaceutical composition (vaccine). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The specification fails to teach how to formulate and use the claimed vaccines. The term "vaccine" encompasses the ability of the specific antigen to induce protective immunity to pathogens that produce infection and disease. The specification does not provide substantive evidence that the claimed vaccines are capable of inducing protective immunity, directed against any binary toxin producing pathogen. This demonstration is required for the skilled artisan to be

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able to use the claimed vaccines for their intended purpose of preventing infections. Without this demonstration, the skilled artisan would not be able to reasonably predict the outcome of the administration of the claimed vaccines, i.e. would not be able to accurately predict if protective immunity has been induced.

The ability to reasonably predict the capacity of a single bacterial immunogen to induce protective immunity from in vitro antibody reactivity studies is problematic. Ellis exemplifies this problem in the recitation that "the key to the problem (of vaccine development) is the identification of the protein component of a virus or microbial pathogen that itself can elicit the production of protective antibodies"(page 572, second full paragraph). Unfortunately, the art is replete with instances where even well characterized antigens that induce an in vitro neutralizing antibody response fail to elicit in vivo protective immunity. See Boslego et al. wherein a single gonococcal pillin protein fails to elicit protective immunity even though a high level of serum antibody response is induced (page 212, bottom of column 2). Cherry, JD (1999, abstract) teaches that a pertussis based vaccine failed, based upon the formulation of the vaccine (see title). Sellman et al (2001, Journal of Biological Chemistry) teach mutants of anthrax protective antigen that evidence inhibited pore forming ability but do not show significant differences from the native wild-type PA (see Table 1, D426A; S428C; K397R; K395C; E275C) and would not predictably serve as a vaccine antigen. Guttman, C (2002) discloses a serious eye condition that developed in two recipients of anthrax vaccine, thus showing negative pathology associated with toxins. Accordingly, the art indicates that it would require undue experimentation to formulate and use a successful vaccine without the prior demonstration of vaccine efficacy.

The specification identifies anthrax protective antigen site specific substitution mutants, and D2L2-deletion mutants that evidence reduced cytotoxicity due to inhibited binding to host cell receptors but the specification fails to provide an adequate written description of how mutant toxin which evidences any mutation that inhibits pore forming ability to any extent, can serve to induce a protective effect in a host. Accordingly, the art indicates that it would require undue

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experimentation to formulate and use a successful vaccine without the prior demonstration of vaccine efficacy.

No art recognized *in vitro* or *in vivo* models are shown in which protection is produced for any and all mutant A-B toxins from the instantly claimed invention. It is clear the mutant toxins compositions are immunogenic but it is not clear that the compositions would result in prevention of infection or disease. No examples containing the missing information are shown.

Given the lack of guidance on how to obtain the desired effect using any B-moiety mutation which inhibits pore forming ability to any extent as a vaccine, and in light of the teachings of the prior art teaching vaccines comprising antigens are unpredictable in methods of treating or preventing infection, the skilled artisan could not make and use the claimed invention. No evidence is of record showing that **any** composition comprising any A-B toxin can or will confer the desired and claimed effect. No working examples are shown which convey the missing information. Therefore, the skilled artisan could not use the claimed compositions to obtain the desired effect of preventing or treating infection without undue experimentation.

14. Claims 4, 5, 19, 20, 30, 31, 34 and 35 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Utilization of abbreviations in the claims is permitted upon definition of the term at their first appearance in the claims. Amendment of claim 4 to specifically define the term "ΔD2L2"

would clearly claim Applicant's invention.

Claims 4 and 19 recite abbreviations that are unclear. Clarification is requested.

Claim 4 depends from claim 1 and recites the species "ΔD2L2". Claim 1 has been

amended to recite the negative limitation "not the deletion of amino acids 302-325". The term

"ΔD2L2" is defined to be amino acids 302-325 in the instant specification. The combination of

W/ 1/2
Amended
recite
OK
Claim 1
Amended
to include
ΔD2L2

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the claim limitations of claim 1 and claim 4 are contradictory and therefore unclear. Claim 4 broadens the scope of claim 1 by adding a species of deletion not encompassed by claim 1.

Claim 5 recites the same negative limitation as amended claim 1. Claim 5 is not further limiting of the independent claim from which it depends.

Claim 19 depends from claim 12 and recites the species "ΔD2L2". Claim 12 has been amended to recite the negative limitation "not the deletion of amino acids 302-325". The "ΔD2L2" species, recited in claim 19, is defined to be amino-acids 302-325 in the instant specification. The combination of the claim limitations of claim 12 and claim 19 are contradictory and therefore unclear. Claim 19 broadens the scope of claim 12 by adding a species of deletion not encompassed by claim 12.

Claim 20 recites the phrase "at least 5 amino acids of the D2L2 loop" and depends from claim 12 which recites the phrase "wherein the mutation is not the deletion of the amino acids 302-325 of anthrax protective antigen SEQ ID No 12. While the negative limitations set forth in claim 12 are clear, the broad recitation of the phrase "at least 5 amino acids of the D2L2 loop" set forth in claim 20, which would include the deletion of the D2L2 loop of SEQ Id No 12. Claim 20 broadens the scope of claim 12 through reciting "at least 5", the upper limit including the entire loop. Claim 12 does not define the A-B toxin to comprise a D2L2 loop, this term recited in claim 20 lacks antecedent basis in claim 12. The invention is not distinctly claimed.

Claim 30 (renumbered) depends from claim 19. Claim 30 defines the mutation to be "a deletion of amino acids 302-325 of the D2L2 Loop" while claim 19 depends from claim 12, which recites the negative limitations "wherein said mutation is not the deletion of the amino acids 302-325 of anthrax protective antigen". The species recited in claim 30 is not set forth in claim 19, thus broadening the scope of claim 19. The claim limitations set forth in renumbered claim 30 are contradictory of the claim limitations set forth in independent claim 12, from which both claims 19 and 30 depend. Claim 30 broadens the scope of both claims 19 and 12. The invention of claim 30 is not distinctly claimed.

Cancelled

Claim 12
amended to
include
ΔD2L2
which
recites
not the
deletion
of amino
acids 302-325

How can
claim 20
include
the deletion
of the D2L2
loop?

oh
claim 12
does not
define
the A-B
toxin to
comprise
a D2L2
loop

partage
the
D2L2
loop

amplifying
which
species
is the

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Claims 31, 34 and 35 (renumbered) all recite the phrase "if said B moiety is anthrax protective antigen". This phrase defines conditional claim limitations. What is the moiety if it is not anthrax protective antigen? Claims 31, 33 and 35 are not further limiting of the base claim from which they depend when the composition is not anthrax protective antigen. Claims 31, 34 and 35 do not distinctly claim Applicant's invention through a positive recitation of claim limitations.

oh
phase
deleted

Claim Rejections - 35 U.S.C. § 102

15. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371© of this title before the invention thereof by the applicant for patent.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) do not apply to the examination of this application as the application being examined was not (1) filed on or after November 29, 2000, or (2) voluntarily published under 35 U.S.C. 122(b). Therefore, this application is examined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

16. Claims 1, 5, 6, 8, 12, 14-15, 20, and 33 (renumbered claim 32) are rejected under 35

U.S.C. 102(e) as being anticipated by Collier et al (US Pat. 5,917,017) filing date June 8, 1994).

~~Collier et al disclose the claimed invention directed to a B moiety of a pore-forming binary~~

A-B toxin, wherein the B-moiety comprises a mutation that inhibits its pore-forming ability (see

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all claims of '017; col. 2, lines 29-44). The reference inherently anticipates the instantly claimed invention.

17. Claims 1-4, 6-8, 12,13, 15, 19-20, 29-31, 33-35 are rejected under 35 U.S.C. 102(e) as being anticipated Cirino et al (US Pat. 6,329,156, filing date March 22, 1999). Cirino et al disclose the claimed invention directed to a B moiety of a pore-forming binary A-B toxin, wherein the B-moiety comprises a mutation that inhibits its pore-forming ability (see col. 10, lines 59-64 and col. 10, lines 1-15, lines 17-58). The reference anticipates the instantly claimed invention.

18. Claims 1, 3, 5, 6, 8, 12, 33 are rejected under 35 U.S.C. 102(b) as being anticipated by Johnson et al (US Pat. 5,792,458). Johnson et al disclose the claimed invention directed to a B moiety of a pore-forming binary A-B toxin, wherein the B-moiety comprises a mutation that inhibits its pore-forming ability (see col. 6, lines 61-67; figures and all claims). The reference anticipates the instantly claimed invention.

19. Claims 1-3, 5-8, 12-18, 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Singh, Y et al (1994, J. Biological Chemistry, Vol. 269). Singh, Y et al (Nov. 1994) discloses the claimed invention directed to a B-moiety of a pore forming binary A-B toxin, wherein the B moiety comprises a mutation that inhibits pore-forming ability.

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The disclosed mutant is a B moiety of anthrax toxin (mutation at residues 313-315) that is unable to form channels (see page 29046, col. 1, paragraph 2-3, Figures 6, page 29043 and page 29044, respectively), and a double mutant that additionally lacks the activation site (mutation at residue positions 164-167, see Table 1, page 29042) for toxicity.

✓ Toxicity correlated with binding of Lethal Factor(LF) was reduced when specific mutants were introduced, but this mutant anthrax toxin was still able to bind Lethal Factor (see Singh et al, abstract, page 29039). Two species of mutant showed a complete lack of toxicity, thus showing no binding to LF (see Figure 1, page 29041). The reference anticipates the instantly claimed invention.

Please Note: ⁴²⁵ The elected species of D~~435~~K is free of the prior art of record. The examiner has chosen a second species to examine from claims 4 and 19, specifically ΔD2L2. The "ΔD2L2" species, recited in claims 4 and 19, is defined to be the deletion of amino acids 302-325 in the instant specification. The following rejection is being applied to not only claims 4 and 19, but also the generic claims that broadly recite any mutation of a B-moiety of an A-B toxin that inhibits pore forming ability.

✓ 20. Claims 1-3, 4, 6-8, 12-20, 29-31 (renumbered claims 28-30), 33-35 are rejected under 35

U.S.C. 102(b) as being anticipated by Miller et al (abstract 712-M, submitted by Applicant in US PTO-1449).

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✓ Miller et al disclose the claimed invention directed to a B moiety of a pore-forming binary A-B toxin, wherein the B-moiety comprises a mutation that inhibits its pore-forming ability (see entire abstract), wherein the resultant B moiety was oligomerized, deficient in the ability to permeabilize membranes, and to translate the enzymatic moieties of anthrax toxin. The B-moiety was in a solution of SDS (dissociating solution, detergent), which is a carrier for moieties. The reference inherently anticipates the instantly claimed invention.

21. Claims 1-3, 5-8, 12-18, 29, 31, 33-35 (renumbered claims 28, 30, 32-34) are rejected under 35 U.S.C. 102(a) as being anticipated by Collier et al (different inventive entity; WO99/42473; submitted by Applicant in US PTO-1449).

✓ Collier et al disclose the claimed invention directed to a B moiety of a pore-forming binary A-B toxin, wherein the B-moiety comprises a mutation that inhibits its pore-forming ability (see entire document and claims), wherein the resultant B moiety was an anthrax protective antigen mutant toxin or the PA63 domain. The moiety was formulated into a prophylactic and immunogenic composition (see page 23, lines 26-28; page 4, lines 17-20). The purified mutant was gel filtered and combined with 150 mM NaCl, a pharmaceutically acceptable carrier (see page 12, lines 17-18). The reference inherently anticipates the instantly claimed invention.

22. Case law applicable to rejections above. Atlas Powder Co. V IRECA, 51 USPQ2d 1943, (FED Cir. 1999) states "Artisans of ordinary skill may not recognize the inherent characteristics

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or functioning of the prior art...However, the discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior art's functioning, does not render the old composition patentably new to the discoverer. "The Court further held that "this same reasoning holds true when it is not a property but an ingredient which is inherently contained in the prior art".

Allowable Subject Matter

23. Claims 4 and 19 recite the elected species directed to "D425K", which is free of the prior art, but are objected to as reciting non-elected inventions, rejected under 35 U.S.C. 112, first and second paragraphs, but would be allowable if the claims were amended to be directed to a mutant anthrax protective antigen mutant of SEQ ID No 21, with the elected substitution.

Conclusion

24. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Anderluh et al (1999) is cited to show (see page 130, Table 1) mutants with reduced permeabilization of sea anemone pore forming toxin. Anu, D et al (2001) is cited to show pore forming ability of *Bacillus thuringiensis* Cry1A mutant toxins. Bossier, F et al (April 2000) is cited to show specific point mutations and in-frame deletions in functional domains of anthrax protective antigen (see entire article). Cabiaux, V et al (1992) is cited to show diphtheria toxin B fragments. De Wölf et al (1994) is cited to show inactive and reactivated mutants and derivatives of B-subunit of cholera toxin. Galen (US Pat. 6,413,768) is cited to show double mutants of the B moiety of Shiga toxin, wherein the mutations were within the active site required for receptor binding, the mutant toxins evidenced reduced toxicity (see col. 23, lines 32-67, col.

Art Unit: 1645

24, lines 1-5 and Table 1). Jobling, MG et al (1991) is cited to show mutant cholera toxins that evidence decreased binding of mutant CT-B to ganglioside GM1 and abolished toxicity (see summary, page 1755). Leppa et al (1999) is cited to show anthrax toxin fusion proteins. Miller et al (1999, Biochemistry; reference provided by Applicant in US PTO-1449) is cited to show mutant PA63 that are inactive pore forming moieties, that are able to form heptamers (see abstract). Moayeri, M et al, (1997) is cited to show substitution mutations that result in reduced toxicity of E.coli hemolysin (see abstract, col. 1, page 2233). Papageorgiou, A et al (1996) is cited to show a mutation in an alpha-2 loop of toxic shock syndrome toxin, wherein the mutation caused the toxin to lose its T-cell mitogenicity and toxicity in experimental animals (abstract). Sellman et al (2001) is cited to show point mutations in anthrax protective antigen that block translocation (see entire document, especially Table 1, page 8375). Steinhorsdottir et al (Jan. 2002) is cited to show mutant beta toxins with reduced binding and pore forming ability (reduced cytotoxicity, see page 46, Figure 1). Singh, Y et al, (April 1999) is cited to show the protective antigen of anthrax toxin to be that portion of the toxin that binds to a cellular receptor. Singh, Y et al (2001) is cited to show a mutant anthrax toxin that inhibits anthrax toxin in vivo. Shatursky, O et al (1999) is cited to show several site specific mutants for a pore forming toxin that is from Clostridium perfringens (see page 294).

25. Tiedemann et al (1995) is cited to show a staphylococcal enterotoxin A mutant that is deficient in MHC class II alpha chain binding, a mutant that is deficient in MHC class II beta chain binding and is unable to form complexes. Welch, RA (2001) is cited to show a review of toxin structure (especially see page 94, top). Wesche, J et al (1998) is cited to show membrane translocation of Lethal factor or edema factor by anthrax protective antigen.

26. Yamaoka, J et al (1997) is cited to show a mutant cholera toxin that has a loss of biological activity through the substitution of Arg for Glu at residue 11 in the beta subunit.

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ginny Portner whose telephone number is (703)308-7543. The examiner can normally be reached on Monday through Friday from 7:30 AM to 5:00 PM except for the first Friday of each two-week period.


If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909. The fax phone number for this group is (703) 308-4242. The Group and/or Art Unit location of your application in the PTO will be Group

Art Unit: 1645

Art Unit 1645. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to this Art Unit.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Vgp December 27, 2002


LYNETTE R. F. SMITH
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

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General information about the entry

Entry name	Q38644
Primary accession number	Q38644
Secondary accession number	Q38566
Entered in TrEMBL in	Release 01, November 1996
Sequence was last modified in	Release 01, November 1996
Annotations were last modified in	Release 19, December 2001

Name and origin of the protein

Protein name	Pore-forming cytotoxin integrase
Synonyms	None
Gene name	INT
From	<u>Bacteriophage phi CTX</u> [TaxID: 35343]
Taxonomy	<u>Viruses; dsDNA viruses, no RNA stage;</u> <u>Caudovirales; Myoviridae.</u>

References

[1] SEQUENCE FROM NUCLEIC ACID.

	<p>MEDLINE=95124301; PubMed=7823914; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Wang Z., Xiong G., Lutz F.;</p> <p>"Site-specific integration of the phage phi CTX genome into the <i>Pseudomonas aeruginosa</i> chromosome: characterization of the functional integrase gene located close to and upstream of attP.";</p> <p>Mol. Gen. Genet. 246:72-79(1995).</p>
[2]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>Hayashi T., Matsumoto H., Ohnishi M., Terawaki Y.;</p> <p>Submitted (OCT-1992) to the EMBL/GenBank/DDBJ databases.</p>
[3]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>Langewische F.W., Balzer A., Lutz F.;</p> <p>Submitted (OCT-1998) to the EMBL/GenBank/DDBJ databases.</p>
[4]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>STRAIN=PHICTX-C;</p> <p>Nakayama K., Hayashi T.;</p> <p>"Whole genome sequence of <i>Pseudomonas aeruginosa</i> cytotoxin-converting phage; phiCTX.";</p> <p>Submitted (OCT-1997) to the EMBL/GenBank/DDBJ databases.</p>
[5]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>STRAIN=PHICTX-C;</p> <p>MEDLINE=90014160; PubMed=2507866; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Hayashi T., Kamio Y., Hishinuma F., Usami Y., Titani K., Terawaki Y.;</p> <p>"<i>Pseudomonas aeruginosa</i> cytotoxin: the nucleotide sequence of the gene and the mechanism of activation of the protoxin.";</p> <p>Mol. Microbiol. 3:861-868(1989).</p>
[6]	<p>SEQUENCE FROM NUCLEIC ACID.</p> <p>STRAIN=PHICTX-C;</p> <p>MEDLINE=93225809; PubMed=8469112; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Hayashi T., Matsumoto H., Ohnishi M., Terawaki Y.;</p> <p>"Molecular analysis of a cytotoxin-converting phage, phi CTX, of <i>Pseudomonas aeruginosa</i>: structure of the attP-cos-ctx region and integration into the serine tRNA gene.";</p>

Mol. Microbiol. 7:657-667(1993).

Comments

None

Cross-references

EMBL	S75107; AAD14164.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	D13409; BAA02675.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	S75107; AAD14165.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	Y13918; CAA74224.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AB008550; [EMBL / GenBank / DDBJ] BAA36272.1; -. [CoDingSequence]
InterPro	IPR002104 ; Phage_integrase. Graphical view of domain structure.
Pfam	PF00589 ; Phage_integrase; 1.
ProDom	[Domain structure / List of seq. sharing at least 1 domain] .
ProtoMap	Q38644 .
PRESAGE	Q38644 .
ModBase	Q38644 .
SWISS-2DPAGE	Get region on 2D PAGE.

Keywords

None

Features

None

Sequence information

Length: 389 AA	Molecular weight: 44431 Da	CRC64: DA98223148071EF1 [This is a checksum on the sequence]
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10	20	30	40	50	60
MADGVEVRGK	RIRIYFRYQG	ELCRESIPGD	ATPENIANAE	RLAGIINYEI	KQGVFSYSRH
70	80	90	100	110	120
FPDSPRVKSN	TLGHYIDLWL	DIKRNQIAAS	GFRGYTSRVE	THIRPRWGDS	QADSIDHLDI
130	140	150	160	170	180
QDWVQNTLMP	KLHNKTVREI	VSNLRQIFRL	YRTRNRSAMD	PTDGIVITLP	DADDPDPFTR
190	200	210	220	230	240
EEIDLILGTE	TARIGELNLA	EFMIWSGPRV	SEAIALAWED	VDLDTGTVVF	RRARVRSQYK
250	260	270	280	290	300
VTKTRRSTRK	VQLLAPALRA	LQQQAKLTRR	LPPVQIEVID	RDNRTKPKQR	VRFVFNHSAS
310	320	330	340	350	360
GAAYSTSDTL	RNGWWHGLR	NAGVRSRGPV	QCRHTFASQM	LSSGIATPEW	IADQMGTST
370	380				
AMIFKHYAKW	ISKDGPDIVG	LLNQALKLS			

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[\[Keywords\]](#) [\[Features\]](#) [\[Sequence\]](#) [\[Tools\]](#)

General information about the entry

Entry name	PAG_BACAN
Primary accession number	P13423
Secondary accession numbers	Q9RQU2 Q9F5R7 Q9KH69
Entered in SWISS-PROT in	Release 13, January 1990
Sequence was last modified in	Release 40, October 2001
Annotations were last modified in	Release 41, June 2002
Name and origin of the protein	
Protein name	Protective antigen [Precursor]
Synonyms	PA PA-83 PA83 Anthrax toxins translocating protein
Contains	PA-20 (PA20) PA-63 (PA63)
Gene name	PAGA or PAG or PXO1-110
From	<u>Bacillus anthracis</u> [TaxID: <u>1392</u>]
Encoded on	Plasmid pXO1.
Taxonomy	<u>Bacteria</u> ; <u>Firmicutes</u> ; <u>Bacillales</u> ; <u>Bacillaceae</u> ; <u>Bacillus</u> .
References	
[1]	SEQUENCE FROM NUCLEIC ACID. MEDLINE=89172073; PubMed=3148491; [<u>NCBI</u> , <u>ExPASy</u> , <u>EBI</u> , <u>Israel</u> , <u>Japan</u>] <u>Welkos S.L.</u> , <u>Lowe J.R.</u> , <u>Eden-McCutchan F.</u> , <u>Vodkin M.</u> , <u>Leppla S.H.</u> , <u>Schmidt J.J.</u> ;
	"Sequence and analysis of the DNA encoding protective antigen of <u>Bacillus anthracis</u> ." <u>Gene</u> 69:287-300(1988).
[2]	SEQUENCE FROM NUCLEIC ACID. STRAIN =28, 33, BA1024, and BA1035;

	<p>MEDLINE=99214082; PubMed=10197996; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Price L.B., Hugh-Jones M., Jackson P.J., Keim P.; "Genetic diversity in the protective antigen gene of <i>Bacillus anthracis</i>."; <i>J. Bacteriol.</i> 181:2358-2362(1999).</p>
[3]	<p>SEQUENCE FROM NUCLEIC ACID. STRAIN=V770-NP1-R / ATCC 14185; MEDLINE=20359347; PubMed=10899854; [NCBI, ExPASy, EBI, Israel, Japan] Cohen S., Mendelson I., Altboum Z., Kobiler D., Elhanany E., Bino T., Leitner M., Inbar I., Rosenberg H., Gozes Y., Barak R., Fisher M., Kronman C., Velan B., Shafferman A.; "Attenuated nontoxinogenic and nonencapsulated recombinant <i>Bacillus anthracis</i> spore vaccines protect against anthrax."; <i>Infect. Immun.</i> 68:4549-4558(2000).</p>
[4]	<p>SEQUENCE FROM NUCLEIC ACID. STRAIN=Sterne; MEDLINE=99445483; PubMed=10515943; [NCBI, ExPASy, EBI, Israel, Japan] Okinaka R.T., Cloud K., Hampton O., Hoffmaster A.R., Hill K.K., Keim P., Koehler T.M., Lamke G., Kumano S., Mahillon J., Manter D., Martinez Y., Ricke D., Svensson R., Jackson P.J.; "Sequence and organization of pXO1, the large <i>Bacillus anthracis</i> plasmid harboring the Anthrax toxin genes."; <i>J. Bacteriol.</i> 181:6509-6515(1999).</p>
[5]	<p>DOMAINS. MEDLINE=91332080; PubMed=1651334; [NCBI, ExPASy, EBI, Israel, Japan] Singh Y., Klimpel K.R., Quinn C.P., Chaudhary V.K., Leppla S.H.; "The carboxyl-terminal end of protective antigen is required for receptor binding and anthrax toxin activity."; <i>J. Biol. Chem.</i> 266:15493-15497(1991).</p>
[6]	<p>CHARACTERIZATION. STRAIN=Sterne; MEDLINE=94327640; PubMed=8051159; [NCBI, ExPASy, EBI, Israel,</p>

	<p><u>Japan]</u> <u>Milne J.C., Furlong D., Hanna P.C., Wall J.S., Collier R.J.;</u> "Anthrax protective antigen forms oligomers during intoxication of mammalian cells."; <u>J. Biol. Chem. 269:20607-20612(1994).</u></p>
[7]	<p>CHARACTERIZATION. MEDLINE=21129592; PubMed=11207581; [<u>NCBI</u>, <u>ExPASy</u>, <u>EBI</u>, <u>Israel</u>, <u>Japan]</u> <u>Beauregard K.E., Collier R.J., Swanson J.A.;</u> "Proteolytic activation of receptor-bound anthrax protective antigen on macrophages promotes its internalization."; <u>Cell. Microbiol. 2:251-258(2000).</u></p>
[8]	<p>TOXIN REGULATION. STRAIN=Weybridge; MEDLINE=94131936; PubMed=8300513; [<u>NCBI</u>, <u>ExPASy</u>, <u>EBI</u>, <u>Israel</u>, <u>Japan]</u> <u>Koehler T.M., Dai Z., Kaufman-Yarbray M.;</u> "Regulation of the Bacillus anthracis protective antigen gene: CO2 and a trans-acting element activate transcription from one of two promoters."; <u>J. Bacteriol. 176:586-595(1994).</u></p>
[9]	<p>MUTAGENESIS OF PHE-342; PHE-343 AND ASP-344. STRAIN=Sterne; MEDLINE=95050722; PubMed=7961869; [<u>NCBI</u>, <u>ExPASy</u>, <u>EBI</u>, <u>Israel</u>, <u>Japan]</u> <u>Singh Y., Klimpel K.R., Arora N., Sharma M., Leppla S.H.;</u> "The chymotrypsin-sensitive site, FFD315, in anthrax toxin protective antigen is required for translocation of lethal factor."; <u>J. Biol. Chem. 269:29039-29046(1994).</u></p>
[10]	<p>MUTAGENESIS OF DOMAIN 4 LOOPS. STRAIN=Sterne; MEDLINE=99185012; PubMed=10085028; [<u>NCBI</u>, <u>ExPASy</u>, <u>EBI</u>, <u>Israel</u>, <u>Japan]</u> <u>Varughese M., Teixeira A.V., Liu S., Leppla S.H.;</u> "Identification of a receptor-binding region within domain 4 of the protective antigen component of anthrax toxin.";</p>

	<u>Infect. Immun. 67:1860-1865(1999).</u>
[11]	<p>MUTAGENESIS OF TRP-375; MET-379 AND LEU-381.</p> <p>STRAIN=Sterne;</p> <p>MEDLINE=21092804; PubMed=11178978; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Batra S., Gupta P., Chauhan V., Singh A., Bhatnagar R.;</p> <p>"Trp 346 and Leu 352 residues in protective antigen are required for the expression of anthrax lethal toxin activity.";</p> <p><u>Biochem. Biophys. Res. Commun. 281:186-192(2001).</u></p>
[12]	<p>MUTAGENESIS OF PHE-581; PHE-583; ILE-591; LEU-595 AND ILE-603.</p> <p>STRAIN=Sterne;</p> <p>MEDLINE=21438996; PubMed=11554763; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Ahuja N., Kumar P., Bhatnagar R.;</p> <p>"Hydrophobic residues Phe552, Phe554, Ile562, Leu566, and Ile574 are required for oligomerization of anthrax protective antigen.";</p> <p><u>Biochem. Biophys. Res. Commun. 287:542-549(2001).</u></p>
[13]	<p>MUTAGENESIS OF PRO-289.</p> <p>STRAIN=Sterne;</p> <p>MEDLINE=21255689; PubMed=11356563; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Khanna H., Chopra A.P., Arora N., Chaudhry A., Singh Y.;</p> <p>"Role of residues constituting the 2beta1 strand of domain II in the biological activity of anthrax protective antigen.";</p> <p><u>FEMS Microbiol. Lett. 199:27-31(2001).</u></p>
[14]	<p>MUTAGENESIS OF GLN-512; ASP-541; LEU-543 AND ARG-621.</p> <p>MEDLINE=21125576; PubMed=11222612; [NCBI, ExPASy, EBI, Israel, Japan]</p> <p>Mogridge J., Mourez M., Collier R.J.;</p> <p>"Involvement of domain 3 in oligomerization by the protective antigen moiety of anthrax toxin.";</p> <p><u>J. Bacteriol. 183:2111-2116(2001).</u></p>
[15]	<p>MUTAGENESIS OF LYS-426; ASP-454 AND PHE-456.</p> <p>MEDLINE=21269403; PubMed=11113126; [NCBI, ExPASy, EBI, Israel, Japan]</p>

	<p><u>Sellman B.R., Nassi S., Collier R.J.;</u> "Point mutations in anthrax protective antigen that block translocation."; J. Biol. Chem. 276:8371-8376(2001).</p>
[16]	<p>MUTAGENESIS OF PRO-213; LEU-216; PHE-231; LEU-232; PRO-234; ILE-236; ILE-239; TRP-255 AND PHE-265. STRAIN=Sterne; MEDLINE=22112896; PubMed=12117959; [NCBI, ExpASY, EBI, Israel, Japan] <u>Chauhan V., Bhatnagar R.;</u> "Identification of amino acid residues of anthrax protective antigen involved in binding with lethal factor."; Infect. Immun. 70:4477-4484(2002).</p>
[17]	<p>X-RAY CRYSTALLOGRAPHY (2.1 ANGSTROMS). MEDLINE=97192099; PubMed=9039918; [NCBI, ExpASY, EBI, Israel, Japan] <u>Petosa C., Collier R.J., Klimpel K.R., Leppla S.H., Liddington R.C.;</u> "Crystal structure of the anthrax toxin protective antigen."; Nature 385:833-838(1997).</p>
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Comments

- **FUNCTION:** One of the three proteins composing the anthrax toxin, the agent which infects many mammalian species and that may cause death. PA binds to a receptor (ATR) in sensitive eukaryotic cells, thereby facilitating the translocation of the enzymatic toxin components, edema factor and lethal factor, across the target cell membrane. PA associated with LF causes death when injected, PA associated with EF produces edema. PA induces immunity to infection with anthrax.
- **SUBUNIT:** Anthrax toxins are composed of three distinct proteins, a protective antigen (PA), a lethal factor (LF) and an edema factor (EF).

None of these is toxic by itself. PA+LF forms the lethal toxin (LeTx); PA+EF forms the edema toxin (EdTx). PA-63 forms heptamers and this oligomerization is required for LF or EF binding. Once activated, at low pH, the heptamer undergoes conformational changes and converts from prepore to pore inserted in the membrane, forming cation-selective channels.

- **SUBCELLULAR LOCATION:** Secreted.
- **DOMAIN:** The molecule is folded into four functional domains. Each domain is required for a particular step in the toxicity process. Domain 1 contains two calcium ions and the proteolytic activation site. Cleavage of the PA monomer releases the subdomain 1a, which is the N-terminal fragment of 20-kDa (PA20). The subdomain 1b is part of the remaining 63-kDa fragment (PA63) and contains the binding sites for LP and EF. Domain 2 is a beta-barrel core containing a large flexible loop that has been implicated in membrane insertion and pore formation. There is a chymotrypsin cleavage site in this loop that is required for toxicity. Domain 3 has a hydrophobic patch thought to be involved in protein-protein interactions. Domain 4 appears to be a separate domain and shows limited contact with the other three domains: it would swing out of the way during membrane insertion. It is required for binding to the receptor; the small loop is involved in receptor recognition.
- **PTM:** Proteolytic activation by furin or a furin-like protease cleaves the protein in two parts, PA-20 and PA-63; the latter is the mature protein. The cleavage occurs at the cell surface and probably in the serum of infected animals as well; both native and cleaved PA are able to bind to the cell receptor. The release of PA20 from the remaining receptor-bound PA63 exposes the binding site for EF and LF, and promotes oligomerization and internalization of the protein.
- **MISCELLANEOUS:** In Ref.9 multiple mutagenesis experiments were performed that showed that the residues present in the small loop of domain 4, and not the ones in the large loop, are involved in receptor recognition.
- **SIMILARITY:** BELONGS TO THE BACTERIAL BINARY TOXIN FAMILY.

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Cross-references

EMBL	M22589; AAA22637.1; [EMBL / GenBank / DDBJ]
	-; [CoDingSequence]
	AF306778; [EMBL / GenBank / DDBJ]
	AAG24446.1; -. [CoDingSequence]
	AF306779; [EMBL / GenBank / DDBJ]
	AAG24447.1; -. [CoDingSequence]
	AF306780; [EMBL / GenBank / DDBJ]
	AAG24448.1; -. [CoDingSequence]
	AF306781; [EMBL / GenBank / DDBJ]
	AAG24449.1; -. [CoDingSequence]
	AF306782; [EMBL / GenBank / DDBJ]
	AAG24450.1; -. [CoDingSequence]
	AF306783; [EMBL / GenBank / DDBJ]
	AAG24451.1; -. [CoDingSequence]
	AF268967; [EMBL / GenBank / DDBJ]
	AAF86457.1; -. [CoDingSequence]
	AF065404; [EMBL / GenBank / DDBJ]
	AAD32414.1; -. [CoDingSequence]
PDB	1ACC; 11-FEB-98. [ExPASy / RCSB]
InterPro	IPR003896 ; Anthrax_toxinB. Graphical view of domain structure.
Pfam	PF03495 ; Binary_toxB; 1.
PRINTS	PR01391 ; BINARYTOXINB.
ProDom	[Domain structure / List of seq. sharing at least 1 domain].
BLOCKS	P13423 .
ProtoNet	P13423 .
ProtoMap	P13423 .
PRESAGE	P13423 .
DIP	P13423 .

ModBase	P13423.
SWISS-2DPAGE	Get region on 2D PAGE.

Keywords

Toxin; Virulence; Calcium-binding; Signal; Plasmid; 3D-structure.

Features

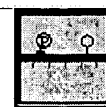
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SIGNAL	<u>1</u>	<u>29</u>	29	
CHAIN	<u>30</u>	<u>764</u>	735	PROTECTIVE ANTIGEN.
CHAIN	<u>30</u>	<u>196</u>	167	PROTECTIVE ANTIGEN, PA-20.
CHAIN	<u>197</u>	<u>764</u>	568	PROTECTIVE ANTIGEN, PA-63.
DOMAIN	<u>30</u>	<u>287</u>	258	DOMAIN 1, CALCIUM-BINDING; LF AND EF BINDING SITES.
DOMAIN	<u>288</u>	<u>516</u>	229	DOMAIN 2, MEMBRANE INSERTION AND HEPTAMERIZATION.
DOMAIN	<u>517</u>	<u>624</u>	108	DOMAIN 3, HEPTAMERIZATION.
DOMAIN	<u>625</u>	<u>764</u>	140	DOMAIN 4, BINDING TO THE RECEPTOR.
CA_BIND	<u>206</u>	<u>206</u>	1	
CA_BIND	<u>208</u>	<u>208</u>	1	
CA_BIND	<u>210</u>	<u>210</u>	1	
CA_BIND	<u>217</u>	<u>217</u>	1	
SITE	<u>196</u>	<u>197</u>	2	CLEAVAGE BY FURIN.
SITE	<u>343</u>	<u>344</u>	2	CHYMOTRYPSIN CLEAVAGE; REQUIRED FOR TRANSLOCATION OF LF AND EF.
VARIANT	<u>560</u>	<u>560</u>		F -> L (IN SVERDLOVSK SAMPLE).
VARIANT	<u>565</u>	<u>565</u>		P -> S (IN STRAIN BA1024).
VARIANT	<u>600</u>	<u>600</u>		A -> V (IN STRAINS BA1024 AND V770-NP1-R).
MUTAGEN	<u>213</u>	<u>213</u>		P->A: DECREASE IN THE ABILITY TO BIND TO LF AND PARTIALLY TOXIC AT HIGH CONCENTRATIONS.
MUTAGEN	<u>216</u>	<u>216</u>		L->A: DECREASE IN THE ABILITY TO BIND TO LF AND PARTIALLY TOXIC AT HIGH CONCENTRATIONS.
MUTAGEN	<u>231</u>	<u>231</u>		F->A: LOSS OF ABILITY TO BIND TO LF AND COMPLETELY NONTOXIC.
MUTAGEN	<u>232</u>	<u>232</u>		L->A: LOSS OF ABILITY TO BIND TO LF AND COMPLETELY NONTOXIC.
MUTAGEN	<u>234</u>	<u>234</u>		P->A: LOSS OF ABILITY TO BIND TO LF AND COMPLETELY NONTOXIC.

MUTAGEN	<u>236</u>	<u>236</u>	I->A: LOSS OF ABILITY TO BIND TO LF AND COMPLETELY NONTOXIC.
MUTAGEN	<u>239</u>	<u>239</u>	I->A: DECREASE IN THE ABILITY TO BIND TO LF AND PARTIALLY TOXIC AT HIGH CONCENTRATIONS.
MUTAGEN	<u>255</u>	<u>255</u>	W->A: NO EFFECT ON LF-BINDING ABILITY AND AS TOXIC AS THE WILD-TYPE.
MUTAGEN	<u>265</u>	<u>265</u>	F->A: NO EFFECT ON LF-BINDING ABILITY AND AS TOXIC AS THE WILD-TYPE.
MUTAGEN	<u>289</u>	<u>289</u>	P->A: REDUCED TOXICITY IN COMBINATION WITH LETHAL FACTOR; DECREASED MEMBRANE INSERTION AND TRANSLOCATION OF THE LETHAL FACTOR.
MUTAGEN	<u>342</u>	<u>342</u>	F->C: LOSS OF TOXICITY PROBABLY DUE TO LOSS OF CAPABILITY TO TRANSLOCATE LF.
MUTAGEN	<u>342</u>	<u>344</u>	FFD->AAA: DECREASE IN TOXICITY PROBABLY DUE TO SLOW TRANSLOCATION OF LF.
MUTAGEN	<u>342</u>	<u>343</u>	MISSING: LOSS OF TOXICITY PROBABLY DUE TO LOSS OF CAPABILITY TO TRANSLOCATE LF.
MUTAGEN	<u>344</u>	<u>344</u>	D->A: DECREASE IN TOXICITY PROBABLY DUE TO SLOW TRANSLOCATION OF LF.
MUTAGEN	<u>375</u>	<u>375</u>	W->A: LOSS OF TOXICITY PROBABLY DUE TO FAULTY MEMBRANE INSERTION OR TRANSLOCATION OF LF/EF INTO THE CYTOSOL.
MUTAGEN	<u>379</u>	<u>379</u>	M->A: NO EFFECT.
MUTAGEN	<u>381</u>	<u>381</u>	L->A: LOSS OF TOXICITY PROBABLY DUE TO FAULTY MEMBRANE INSERTION OR TRANSLOCATION OF LF/EF INTO THE CYTOSOL.
MUTAGEN	<u>426</u>	<u>426</u>	K->A: LOSS OF CAPABILITY TO UNDERGO CONFORMATIONAL CHANGES THAT LEAD TO PORE FORMATION AND TRANSLOCATION.
MUTAGEN	<u>454</u>	<u>454</u>	D->A: LOSS OF CAPABILITY TO UNDERGO CONFORMATIONAL CHANGES THAT LEAD TO PORE FORMATION AND TRANSLOCATION.
MUTAGEN	<u>456</u>	<u>456</u>	F->A: LOSS OF CAPABILITY TO UNDERGO CONFORMATIONAL CHANGES THAT LEAD TO PORE FORMATION AND TRANSLOCATION.
MUTAGEN	<u>512</u>	<u>512</u>	Q->A: LOSS OF HEPTAMERIZATION CAPABILITY.
MUTAGEN	<u>541</u>	<u>541</u>	D->A: LOSS OF HEPTAMERIZATION CAPABILITY.
MUTAGEN	<u>543</u>	<u>543</u>	L->A: DECREASE IN HEPTAMERIZATION CAPABILITY.
MUTAGEN	<u>581</u>	<u>581</u>	F->A: LOSS OF TOXICITY DUE TO DEFECTIVE OLIGOMERIZATION.
MUTAGEN	<u>583</u>	<u>583</u>	F->A: DECREASE IN TOXICITY DUE TO DEFECTIVE

			OLIGOMERIZATION.
MUTAGEN	<u>591</u>	<u>591</u>	I->A: LOSS OF TOXICITY DUE TO DEFECTIVE OLIGOMERIZATION.
MUTAGEN	<u>595</u>	<u>595</u>	L->A: LOSS OF TOXICITY DUE TO DEFECTIVE OLIGOMERIZATION.
MUTAGEN	<u>603</u>	<u>603</u>	I->A: LOSS OF TOXICITY DUE TO DEFECTIVE OLIGOMERIZATION.
MUTAGEN	<u>621</u>	<u>621</u>	R->A: NO EFFECT.
CONFLICT	<u>314</u>	<u>314</u>	Q -> E (IN REF. <u>1</u>).
STRAND	<u>49</u>	<u>53</u>	5
TURN	<u>56</u>	<u>57</u>	2
STRAND	<u>61</u>	<u>66</u>	6
STRAND	<u>71</u>	<u>71</u>	1
STRAND	<u>74</u>	<u>74</u>	1
HELIX	<u>76</u>	<u>78</u>	3
TURN	<u>80</u>	<u>81</u>	2
HELIX	<u>84</u>	<u>87</u>	4
STRAND	<u>91</u>	<u>99</u>	9
STRAND	<u>104</u>	<u>110</u>	7
TURN	<u>111</u>	<u>112</u>	2
HELIX	<u>113</u>	<u>115</u>	3
STRAND	<u>116</u>	<u>120</u>	5
TURN	<u>121</u>	<u>122</u>	2
STRAND	<u>123</u>	<u>126</u>	4
STRAND	<u>135</u>	<u>137</u>	3
TURN	<u>139</u>	<u>140</u>	2
STRAND	<u>142</u>	<u>150</u>	9
STRAND	<u>159</u>	<u>159</u>	1
STRAND	<u>162</u>	<u>166</u>	5
STRAND	<u>172</u>	<u>174</u>	3
TURN	<u>177</u>	<u>179</u>	3
STRAND	<u>180</u>	<u>181</u>	2
TURN	<u>207</u>	<u>208</u>	2
HELIX	<u>214</u>	<u>219</u>	6
STRAND	<u>221</u>	<u>225</u>	5
STRAND	<u>230</u>	<u>234</u>	5



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HELIX	<u>237</u>	<u>241</u>	5
TURN	<u>242</u>	<u>244</u>	3
STRAND	<u>248</u>	<u>248</u>	1
TURN	<u>252</u>	<u>253</u>	2
TURN	<u>257</u>	<u>258</u>	2
HELIX	<u>264</u>	<u>269</u>	6
TURN	<u>270</u>	<u>270</u>	1
TURN	<u>274</u>	<u>275</u>	2
HELIX	<u>278</u>	<u>281</u>	4
TURN	<u>283</u>	<u>284</u>	2
STRAND	<u>285</u>	<u>285</u>	1
STRAND	<u>291</u>	<u>302</u>	12
STRAND	<u>318</u>	<u>326</u>	9
STRAND	<u>331</u>	<u>331</u>	1
STRAND	<u>350</u>	<u>350</u>	1
STRAND	<u>357</u>	<u>363</u>	7
TURN	<u>383</u>	<u>384</u>	2
STRAND	<u>387</u>	<u>397</u>	11
STRAND	<u>403</u>	<u>403</u>	1
STRAND	<u>410</u>	<u>414</u>	5
TURN	<u>415</u>	<u>417</u>	3
STRAND	<u>418</u>	<u>423</u>	6
TURN	<u>427</u>	<u>428</u>	2
STRAND	<u>434</u>	<u>434</u>	1
TURN	<u>436</u>	<u>437</u>	2
STRAND	<u>438</u>	<u>440</u>	3
TURN	<u>443</u>	<u>444</u>	2
STRAND	<u>448</u>	<u>449</u>	2
TURN	<u>456</u>	<u>457</u>	2
STRAND	<u>461</u>	<u>463</u>	3
HELIX	<u>465</u>	<u>474</u>	10
STRAND	<u>476</u>	<u>481</u>	6
STRAND	<u>487</u>	<u>492</u>	6

TURN	<u>493</u>	<u>496</u>	4
STRAND	<u>497</u>	<u>505</u>	9
HELIX	<u>506</u>	<u>516</u>	11
STRAND	<u>517</u>	<u>522</u>	6
TURN	<u>524</u>	<u>526</u>	3
STRAND	<u>530</u>	<u>535</u>	6
STRAND	<u>537</u>	<u>537</u>	1
STRAND	<u>551</u>	<u>551</u>	1
HELIX	<u>552</u>	<u>560</u>	9
STRAND	<u>563</u>	<u>563</u>	1
TURN	<u>565</u>	<u>566</u>	2
STRAND	<u>570</u>	<u>571</u>	2
TURN	<u>572</u>	<u>573</u>	2
STRAND	<u>574</u>	<u>575</u>	2
HELIX	<u>576</u>	<u>578</u>	3
STRAND	<u>579</u>	<u>583</u>	5
HELIX	<u>585</u>	<u>598</u>	14
TURN	<u>599</u>	<u>599</u>	1
TURN	<u>603</u>	<u>603</u>	1
HELIX	<u>604</u>	<u>609</u>	6
STRAND	<u>611</u>	<u>611</u>	1
STRAND	<u>613</u>	<u>613</u>	1
TURN	<u>614</u>	<u>615</u>	2
STRAND	<u>617</u>	<u>622</u>	6
TURN	<u>623</u>	<u>624</u>	2
STRAND	<u>626</u>	<u>627</u>	2
TURN	<u>629</u>	<u>630</u>	2
STRAND	<u>633</u>	<u>635</u>	3
HELIX	<u>638</u>	<u>643</u>	6
TURN	<u>644</u>	<u>645</u>	2
STRAND	<u>648</u>	<u>651</u>	4
STRAND	<u>655</u>	<u>658</u>	4
HELIX	<u>662</u>	<u>666</u>	5

TURN	<u>667</u>	<u>667</u>	1
STRAND	<u>668</u>	<u>676</u>	9
TURN	<u>678</u>	<u>679</u>	2
STRAND	<u>682</u>	<u>684</u>	3
TURN	<u>685</u>	<u>686</u>	2
TURN	<u>689</u>	<u>690</u>	2
STRAND	<u>695</u>	<u>697</u>	3
TURN	<u>699</u>	<u>700</u>	2
STRAND	<u>703</u>	<u>706</u>	4
TURN	<u>709</u>	<u>713</u>	5
TURN	<u>721</u>	<u>722</u>	2
STRAND	<u>724</u>	<u>731</u>	8
HELIX	<u>732</u>	<u>734</u>	3
TURN	<u>748</u>	<u>749</u>	2
STRAND	<u>753</u>	<u>759</u>	7
HELIX	<u>760</u>	<u>764</u>	5

Sequence information

Length: 764 AA [This is the length of the unprocessed precursor]	Molecular weight: 85810 Da [This is the MW of the unprocessed precursor]	CRC64: 3AE1EFBF48FAA03F [This is a checksum on the sequence]
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10	20	30	40	50	60
MKKRKVLIP	L MALSTILVSS	TGNLEVIQAE	VKQENRLLNE	SESSSQGLLG	YYFSDLNFQA
70	80	90	100	110	120
PMVVTSSSTG	DLSIPSSELE	NIPSENQYFQ	SAIWSGFIKV	KKSDEYTFAT	SADNHVTMWV
130	140	150	160	170	180
DDQEVINKAS	NSNKIRLEKG	RLYQIKIQYQ	RENPTKGLD	FKLYWTDSON	KKEVISSDNL
190	200	210	220	230	240
QLPELKQKSS	NSRKKRSTSA	GPTVPDRDND	GIPDSLEVEG	YTVDVKNKRT	FLSPWISNIH
250	260	270	280	290	300
EKKGLTKYKS	SPEKWSTASD	PYSDFEKVTG	RIDKNVSPEA	RHPLVAAYPI	VHVDMENIIL
310	320	330	340	350	360
SKNEDQSTQN	TDSQTRTISK	NTTSRTHTS	EVHGNAEVHA	SFFDIGGSVS	AGFSNSNSST
370	380	390	400	410	420
VAIDHSLSLA	GERTWAETMG	LNTADTARLN	ANIRYVNTGT	APIYNVLPTT	SLVLGKNQTL
430	440	450	460	470	480
ATIKAKENQL	SQILAPNNYY	PSKNLAPIAL	NAQDDFSSTP	ITMNYNQFLE	LEKTKQLRLD
490	500	510	520	530	540
TDQVYGNIAI	YNFENGVRV	DTGSNWSEVL	PQIQETTARI	IFNGKDLNLV	ERRIAAVNPS
550	560	570	580	590	600
DPLETTKPD	M TLKEALKIAF	GFNEPNGNLQ	YQKGDITEFD	FNFDQQTSON	IKNQLAELNA
610	620	630	640	650	660
TNIYTVLDKI	KLNAMNILI	RDKRFHYDRN	NIAVGADSV	VKEAHREVIN	SSTEGLLNI
670	680	690	700	710	720
DKDIRKILSG	YIVEIEDTEG	LKEVINDRYD	MLNISSLRQD	GKTFIDFKKY	NDKLPLYISN
730	740	750	760		
PNYKVNVA	Y TKENTIINPS	ENGDTSTNGI	KKILIFSKKG	YEIG	

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